Express Mail Label No.: EV058165096US
Date of Deposit: April 23, 2002

Attorney Docket No. 17811-014 CIP (M-14 CIP)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Hunziker *et al.*		
SERIAL NUMBER:	09/546,269	EXAMINER:	J. Witz
FILING DATE:	April 10, 2000	ART UNIT:	1651
FOR:	IMPROVED KERATINOCYTE CULTURE AND USES THEREOF		

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

I, ALAIN LIMAT, hereby declare and state as follows:

1. I received my diploma in biochemistry in 1975 as well as my Ph.D. degree in 1979 at the University of Fribourg/Switzerland and my privat docent (habilitation degree) in 1997 at the University of Bern/Switzerland. I am a named inventor on this application. I have been working in the field of cell and molecular biology and with methods of treating skin defects since 1982.

2. I understand that the pending claims are directed to methods for the selection of keratinocyte precursor cells from the outer root sheath of hair for subsequent use in a composition for healing a skin defect.

3. I am aware of the Examiner's October 23, 2001 Office Action. In particular, I understand that the Examiner has rejected the pending claims under 35 U.S.C. § 103(a) contending that the pending claims are obvious in view of United States Patent 5,968,546 to Baur et al. in combination with Lenoir et al. and Lenoir-Viale et al. According to the Examiner, "the patent to Baur teaches that the plucked hair is not cultured in both but instead is subject to dissection and the hair bulb and infundibular parts are removed and the follicles are subsequently cultured." (Office Action at page 2). The Examiner also notes that Lenoir et al.

and Lenoir-Viale et al. disclose the production of epidermal equivalents from human hair follicles and that these references teach that the removal of the hair bulb is done merely as a matter of convenience. The Examiner further alleges that the dissection step of Baur is not critical to the production of epidermal or skin equivalents.

4. I make this declaration to rebut the Examiner's rejection, with which I do not agree. I understand that the claims of the instant application have been amended to specify that, in addition to requiring the use of an intact hair follicle, the methods also require that the primary and organotypic cultures contain human serum in a concentration of less than 5% and that the keratinocyte precursor cells are seeded at a density of between $3x10^4$ cells cm^2 and $1x10^5$ cells cm^2. In my opinion, such methods are neither taught nor suggested by the combination of Baur, Lenoir, and Lenoir-Viale. Additionally, it is my opinion that the claimed methods for the selection of keratinocytes precursor cells are improvements over the methods disclosed by Baur. Moreover, the claimed methods would not be obvious to the ordinarily skilled artisan with knowledge of Baur, Lenoir, and Lenoir-Viale for at least the following reasons.

5. There are several advantages to using an intact hair follicle rather than further dissecting the hair follicle prior to culturing (as done in the methods of Baur). Contrary to the Examiner's contention, I believe that the ordinarily skilled artisan would not have been motivated to modify the process disclosed in Baur by eliminating the dissection step. As noted in the specification, we were the first to realize that such additional dissection was not required in order to generate epidermal or skin equivalents according to the invention. (*See* page 3, lines 18-21). I also note that the discussion of this dissection in Lenoir and Lenoir-Viale would not have motivated the ordinarily skilled artisan to modify the teaching of Baur to arrive at the claimed methods.

6. By eliminating this additional step, Applicants have not only simplified the production of epidermal or skin equivalents, but they have also made the entire production process less expensive and less time consuming. Moreover, the production process, without this dissection step, is also less labor-intensive. All of these advantages result in methods that are, in my

opinion, superior to those of Baur, resulting in a saving of time of 50% of the hair follicle preparation step.

7. The methods disclosed in the instant application require that the primary and organotypic cultures contain less than 5% serum. In contrast, the methods disclosed in the examples in the Baur patent use culture media containing 10% serum. In my opinion, the use of a lower serum concentration contributes to the production of an improved epidermal or skin equivalents.

8. Specifically, by lowering the concentration of serum, we were able to reduce the production costs associated with the claimed methods of making epidermal or skin equivalents, resulting in a cost reduction of the culture medium of 25% or more.

9. Moreover, lower serum concentrations also help to reduce the risks of disease transmission associated with the use of clinical blood products. This, in turn, is important to the acceptance of transplantation procedures using epidermal or skin equivalents prepared according to the methods of the instant invention.

10. I also note that lower serum concentrations help to improve the state of the cells in the equivalents. As noted in the specification, "[a] normal stratified epidermis consists of a basal layer of small cuboidal cells, several spinous layers of progressively flattened cells, a prominent granular layer and an orthokeratotic horny layer." (Specification at page 5, lines 27-29). Each of these layers have been detected in the epidermal equivalents made according to the methods of the present invention. (*See* id. at lines 29-30). Likewise, "[l]ocalization of those epidermal differentiation products that have been assayed by immunohistochemistry (e.g. keratin, involucrin, filaggrin, integrins) is identical to that found in normal epidermis." (Specification at page 5, line 30 through page 6, line 3). This improved stratification leads to an epidermal or skin equivalent that closely resembles natural skin.

11. The use of 5% or less of human serum in the production of the epidermal or dermal equivalents results in better stratification of the cells. In contrast, when higher serum concentrations are employed (such as the 10% concentration used in Baur), less stratification of the epidermal equivalents is observed. Thus, production of the equivalents according to the methods of the invention results in a more normalized epidermal situation than that of Baur. In other words, epidermal or skin equivalents prepared using the lower serum concentrations more closely resemble normal epithelium. This, in turn, enhances the success rate of transplantation procedures using the epidermal or skin equivalents of the invention.

12. Therefore, it is my opinion that, for this reason, the epidermal or skin equivalents prepared according to the methods of the instant invention are superior to those prepared according to the methods disclosed in Baur. Moreover, there is no indication in Baur that a lower serum concentration would be useful or beneficial to the production method.

13. I also note that the claimed methods now specify that for organotypically-culturing the keratinocyte precursor cells, the keratinocyte precursor cells are seeded at a density between 3×10^4 cells cm^2 and 1×10^5 cells cm^2. Our research has indicated that at serum concentrations of less than 5%, this is the optimal inoculation density for preparing the epidermal or skin equivalents according to the methods of the instant invention. When other keratinocyte precursor cell inoculation densities are employed, a longer period of time is required for the cell culture to reach confluence. Here, an important condition for organotypic culture is the maintenance of the cells at the air-liquid interface. (*See* Specification at page 6, lines 16-18). The use of this specific cell density results in a faster time to achieve confluence, *i.e.* a faster time to achieve the important "lifted" culturing condition. By optimizing the inoculation density the total culture period is reduced by 2-3 days. Therefore, it is my opinion that the use of this culture density helps to streamline the production of the epidermal or skin equivalents according to the methods of the instant invention.

14. In my opinion, the combination of these parameters (intact hair follicles, les than 5% human serum, and keratinocyte precursor cells seeded at a density of between 3×10^4 cells cm^2

and $1x10^5$ cells cm^2) yields epidermal or skin equivalents that are superior to those prepared according to the methods of Baur. Any one of these parameters alone is not sufficient to produce such superior epidermal or dermal equivalents. Rather, the claims now specifically require all of these parameters in combination to produce such equivalents. The combination of these parameters is not taught or suggested by the combination of Baur, Lenoir, and or Lenoir-Viale. Thus, for all the foregoing reasons, I believe that the Examiner should withdraw this rejection and allow the pending claims.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Alain Limat

Signed at Lausanne, SWITZERLAND
this 19 day of April 2002

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